De provitaminen-D van de mossel (Mytilus edulis)

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SUMMARY.

The sterol mixture of the mussel (Mytilus edulis) contains a provitamin-D which is converted by ultraviolet irradiation into an antirachitic active product, equalling in chicken-rat ratio a good grade of codliver oil; we have investigated the composition of this „mussel provitamin-D".

Boer, van Niekerk, Reerink and van Wijk have already discovered that the optically pure provitamin, which was isolated by means of the chromatographic adsorption method, can be split up in two parts, one of them (called by us substance K) representing the high chicken-activity of the irradiated mussel provitamin; the other part has, after irradiation, a very low chicken-rat ratio, equalling that of calciferol.

This component was purified by Boer et al. to a product with constant physical, chemical and biological properties (called by us substance R). This substance R has a somewhat higher molecular weight than ergosterol and contains three double bonds; the irradiation product has half the antirachitic activity (rat-assay) of an in the same manner prepared irradiation product of ergosterol.

These and the other properties do not agree with those of any of the hitherto known provitamins-D; however the possibility that substance R is a mixture of closely related sterols could not be precluded.

Continuing this we investigated not the mussel provitamin itself but the irradiation product from which we had removed the not photochemically converted part in the usual way.

By means of the 3,5-dinitro-benzoic-acid esters we succeeded in separating from this irradiation product vitamin-D_3 which was characterized by the 3,5-dinitro-benzoic-acid ester, the p-nitro-benzoic-acid ester and the molecular compound with cholesterol; these compounds were identified with the corresponding derivatives of vitamin-D_3, prepared from an irradiation product of 7-dehydrocholesterol.

In addition to this we isolated an apparently pure antirachitic active product which we called D_x, having the following constant properties.

The absorption spectrum is practically identical with that of calciferol (maximum extinction 97 % of that of calciferol). D_x contains four double bonds, m.p. 108—109° C, $[\alpha]_D^{25} = +103°$ (acetone); 3,5-dinitro-benzoic-acid ester: m.p. 138—139° C, $[\alpha]_D^{25} = +96°$ (acetone), $[\alpha]_D^{26} = +106°$ (chloroform). D_x possesses an antirachitic activity of 20.000 I.U./mg (rat-assay) and is not or rather not chick-active. The analyses correspond with $C_{28}H_{44}O$ or $C_{29}H_{46}O$.

However by ozonolysis substance D_x proved to be a mixture; steam destillation of the reaction product yielded a mixture of aldehydes (possibly aldehyde + ketone), the analyses showing a composition between $C_6H_{12}O$ and $C_7H_{14}O$; the 2,4-dinitrophenylhydrazones could not be separated.

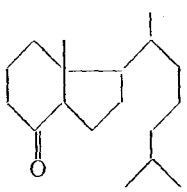
Upon esterification of D_x with trimethylacetic acid we succeeded in separating from the mixture the trimethylacetic-acid ester of calciferol.

The data obtained lead us to the opinion that D_x consists of a mixture of about equal amounts of calciferol and of an antirachitically rather inactive substance having the constitution $C_{28}H_{44}O$ or $C_{29}H_{46}O$ and possessing an unsaturated side-chain.

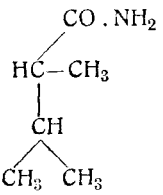
The described „provitamin" substance R corresponds probably with the „vitamin" D_x; substance K can be concluded to be 7-dehydro-cholesterol.

By ozonolysis of the complete irradiation product, from which only the photochemically inconverted material was removed, the composition of the mussel provitamin could be further elucidated.

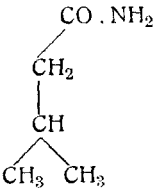
The volatile degradation-aldehydes from the side chains were oxidized by means of ammoniacal silver solution to the corresponding acids and again these were converted into the acid-amides. We obtained the following degradation products: formaldehyde (resulting from the methylene group at C_{10}), the ketone $C_{18}H_{32}O$ from vitamin-D_3, methyl-isopropyl-acetamide (resulting from the irradiation products of ergo-sterol) and isopropyl-acetamide.



ketone $C_{18}H_{32}O$

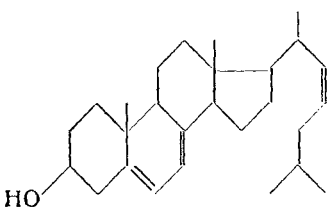


methyl-isopropyl-acetamide



isopropyl-acetamide

The latter degradation product leads us to the opinion that the mussel provitamin contains the hitherto unknown „provitamin-D" cholestatriene-5,7,22-ol-3.



cholestatriene-5,7,22-ol-3

From a quantitative consideration of the degradation products, as compared with degradations of calciferol and the irradiation product of 7-dehydro-cholesterol, as well as from biological and

other data, we conclude to the following composition of the mussel provitamin-D:

7-dehydro-cholesterol	about	$^3/_6$ parts
ergosterol	„	$^1/_6$ „
cholestatriene-5,7,22-ol-3	„	$^2/_6$ „

As fourth component we suppose to be present, in minor quantity, the „provitamin-D" corresponding with the second component of substance D_x (28 or 29 C-atoms; unsaturated side-chain).

The present amounts of 7-dehydro-cholesterol and ergosterol account for the total antirachitic activity of irradiated mussel provitamin; so we may conclude that the supposed cholestatriene-5,7,22-ol-3 should not or rather not be activatable, this conclusion however has to be checked by synthesis.

Upon application of our degradation method to mussel sterol from which the provitamin had been removed, we obtained a mixture of acid-amides.

This mixture had the same analytical composition (between $C_5H_{11}ON$ and $C_6H_{13}ON$) as the mixture obtained by degradation of the irradiated mussel provitamin, from which we isolated, as described, methyl-isopropyl-acetamide and isopropyl-acetamide.

Stevens obtained from the same part of the mussel sterol cholesterol and brassicasterol (unpublished).

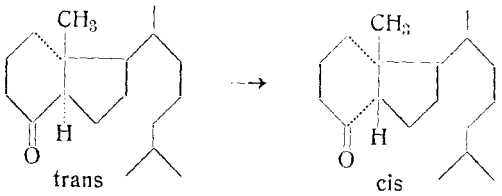
These investigations, details of which will be published at the time, lead us to the attractive assumption that the mussel sterol (provitamin-free part), broadly outlined, should be composed of:

cholesterol
brassicasterol
cholestadiene-5,22-ol-3 } round about half of total composition
stigmasterol?

So the difference in provitamin and other part should mainly consist of a difference in degree of saturation.

The semicarbazone of the ketone $C_{18}H_{32}O$ (degradation product of vitamin-D_3) was found by us to be isomerized, by warming it with a solution of semicarbazide-acetate, to a semicarbazone possessing a lower melting point, lower rotation and greater solubility. This isomerization takes place with the ketone itself by warming it with dilute sulfuric acid.

We suppose this to be caused by conversion of the trans-hydrindane into the cis-hydrindane structure;



in accordance with the explanation given by Dimroth for similar isomerizations.